

EFFECT OF IONIZING RADIATION ON BEEF BOLOGNA CONTAINING SOY PROTEIN CONCENTRATE

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ABSTRACT

*Soy protein concentrate (SPC), an extender, is a common additive in ready-to-eat (RTE) meat products. SPC contains antioxidants that could potentially interfere with the ability of ionizing radiation to eliminate *Listeria monocytogenes* from RTE meat products. When *L. monocytogenes* was inoculated into cooked beef bologna emulsion containing 0, 1.75, or 3.5% SPC the gamma radiation D_{10} values, at radiation doses of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 kGy, were 0.66, 0.68, and 0.71 kGy, respectively. Soluble antioxidant power, as determined by the Ferric Reducing Antioxidant Power (FRAP) assay was 1958, 3572, and 5494 mol in bologna emulsion containing 0, 1.75 and 3.5% SPC, respectively. Soluble antioxidant power was not affected by ionizing radiation. SPC did not prevent ionizing radiation induced lipid oxidation as determined by Thiobarbituric Acid Reactive Substance (TBARS) assay. Hunter color analysis of both unirradiated and irradiated bologna slices containing SPC indicated decreased a-value as a result of irradiation, while the addition of SPC helped maintain b-value and L-value. The inclusion of SPC did not represent a barrier to ionizing radiation pasteurization of fine emulsion sausages for the parameters examined.*

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INTRODUCTION

Listeria monocytogenes is a foodborne pathogen capable of growth at refrigerated temperatures and in high salt environments (Smith 1996). It is a frequent postprocess contaminant of ready-to-eat (RTE) meat products (Nickelson and Schmidt 1999). A number of foodborne illness outbreaks and recalls have been attributed to *L. monocytogenes* (Anon. 1998; Barnes *et al.* 1989; CDC 1989). Because of the high mortality rate associated with listeriosis, approximately 20% in susceptible populations, *L. monocytogenes* is given zero tolerance in ready-to-eat meat products in the United States (Mead *et al.* 1999; USDA 1989).

Ionizing radiation can eliminate *L. monocytogenes* from RTE meat products; however, the radiation resistance of *L. monocytogenes* can vary with product (Sommers and Thayer 2000). Soy protein concentrate (SPC), an extender, is used in the manufacture of fine emulsion sausage including bologna and frankfurters (Ockermann 1989; 9CFR318.7). SPC also possesses antioxidants (Ho *et al.* 1995; Romjin *et al.* 1991). Antioxidants can sometimes increase the radiation resistance of foodborne pathogens by scavenging radiolytic products of water (Sharma *et al.* 2000; Kim and Thayer 1995).

This work addressed the following questions: (1) What is the effect of SPC on the D_{10} value (the radiation dose required to reduce the viable organism population by 90%) of *L. monocytogenes* inoculated onto cooked beef bologna emulsion containing SPC? (2) What is the effect of SPC, in combination with ionizing radiation, on the antioxidant power of the bologna emulsion? (3) What is the effect of SPC, in combination with ionizing radiation, on lipid oxidation in the bologna emulsion? and (4) What affect does SPC and ionizing radiation have on bologna color?

MATERIALS AND METHODS

Cured Meat Manufacture

In order to maintain control over formulation and manufacturing practices all emulsions were produced in house. Standard procedures and formulations were used (Rust 1976; Ockerman 1989). Ground beef (15% fat) was emulsified in a Hobart Model HCM40 Cutter-Mixer. Cure ingredients and additives (w/w per kg meat) included 3% sodium chloride, 3% dextrose, 0.5 % sodium tripolyphosphate, 0.05% sodium erythorbate, 200 mg/kg sodium nitrite, and 20% added water. Spices were not added due to the variability of commercial formulations and to limit the experimental variables in the study. MRS 6:1 soy protein concentrate (Bavaria Corp., Occacopee, FL) was added to obtain concentrations of 0, 1.75, and

3.5%. Concentrations of SPC above 3.5% were not utilized due to the thickness of the emulsion at higher concentrations. The emulsion was stuffed into 1¾" fibrous casings (Dewied Int., Santa Fe, NM) and cooked in a Koch Model KL-50 Smokehouse (Koch Inc., Kansas City, MO) to an internal product temperature of 73C. The dry bulb setting was 90C and wet bulb setting was 63C for a relative humidity of approximately 47%.

After the internal temperature was reached the bologna was chilled using a sterile cold water bath, the casings removed (aseptically), and the bologna placed in No. 400 Stomacher bags (Tekmar, Inc., Cincinnati, OH). The meats were vacuum-packed to 0.26-mm Hg using a Multi-Vac A300 Vacuum Packager (Kansas City, MO). They were then overpacked in Mil-B-131-H Foil bags (Bell Fibre Products Corp., Columbus, GA) and stored at 0 to 2C until use. Background microbiota, monitored by pour plate assay (see below), was less than 2.0 log₁₀ CFU/gram.

Strains

Four *L. monocytogenes* isolates (7644, 15313, 43256, 49594) were obtained from the American Type Culture Collection (Manassas, VA). The strains were propagated on Tryptic Soy Agar (Difco Laboratories, Detroit, MI) at 37C and maintained at 0 to 2C until use. Identity as *Listeria* was confirmed by Gram stain followed by analysis with Gram-positive Identification (GPI) cards using the Vitek Automicrobic System (bioMerieux Vitek, Inc., Hazelwood, MO).

Bacterial Cultures

Each *L. monocytogenes* strain was cultured independently in 100-mL Tryptic Soy Broth (Difco Laboratories, Detroit, MI) in a baffled 500-mL Erlenmeyer culture flask at 37C (150 rpm) for 18 h. The cultures were then combined and the mixture sedimented by centrifugation (1725 x g for 30 min). *L. monocytogenes* was then concentrated, as a cocktail, ten-fold by resuspension in 40-mL of Butterfield's phosphate buffer (BPB) (Applied Research Institute, Newtown, CT).

L. monocytogenes Survival Curves

For emulsion experiments 50 g of bologna was emacerated and placed in a No. 400 Stomacher bag (Tekmar Co., Cincinnati, OH), inoculated with 5-mL *L. monocytogenes* cocktail, and mixed by stomaching for 90 s. Aliquots (5 g) were then placed in No. 400 stomacher bags, vacuum-packed to 0.26-mm Hg using a

Multi-Vac Model A300 packager (Multi-Vac, Kansas City MO), and stored at 0-2°C to await irradiation (30–60 min).

Gamma Irradiation

A Lockheed Georgia Company (Marietta, GA) self-contained ^{137}Cs gamma irradiation source was used. The radiation source consisted of 23 individually sealed source pencils placed in an annular array. The 22.9×63.5 cm cylindrical sample chamber was located central to the array when placed in the operating position. The inoculated vacuum-packed samples were placed vertically in the sample chamber to insure uniformity of dose.

The dose rate provided by the irradiator was 0.10 kGy/min. The temperature during irradiation process was maintained at $4.0(\pm 1.0)^\circ\text{C}$ by the gas phase of a liquid nitrogen source which was introduced directly into the top of the sample chamber. The temperature was monitored during the entire irradiation process using two thermocouples placed on the side of the sample bags. The dose delivered was verified by use of 5-mm alanine pellet dosimeters, which were then measured using a Bruker EMS 104 EPR Analyzer (Billerica, MA). Radiation doses used were 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 kGy.

Plate Counts

The samples were assayed using standard pour plate procedures. Five-gram samples were diluted in 45-mL BPB and mixed by stomaching for 90 s. The samples were then serially diluted in BPB, using ten-fold dilutions, and 1 mL of diluted sample pour plated using Tryptic Soy Agar. Three 1-mL aliquots were plated per dilution. The plates were then incubated for 48 to 72 h at 37°C prior to scoring.

D_{10} -Values. D_{10} is defined as the radiation dose required to produce a 90% reduction in viable organisms. The average CFU/plate of an irradiated sample (N) was divided by the average CFU/plate of the untreated control (N_0) to produce a survivor ratio (N/N_0). D_{10} value was determined by calculating the reciprocal of the slope provided by the (N/N_0) ratios (Sommers and Thayer 2000). Each experiment was conducted independently four times. The log reduction data from the 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 kGy doses were used for D_{10} value determination.

Ferric Reducing Antioxidant Power (FRAP) Assay. Five-gram aliquots of bologna emulsion with and without the soy protein concentrate were vacuum packaged in No. 400 Stomacher bags, overpacked in Mil-B foil bags as previously described, and irradiated to ionizing radiation doses of 0, 2.0, and 4.0 kGy. Following irradiation the samples were diluted 1/10 in sterile deionized water, emacerated, and mixed by stomaching for 90 s. Aliquots (0.05 mL) were used for determination of antioxidant power. Total antioxidant power of the cured meat ($n = 15$) was measured directly by FRAP assay (Benzie and Strain 1999). In the assay antioxidants reduce ferric tripyridyltriazine at low pH to the ferrous form, which has an intense blue color. Absorbance was measured at 593 nm and concentration calculated against a standard curve of ferrous iron sulfate. FRAP values are expressed as mol/g antioxidant.

Lipid Oxidation

Bologna emulsion (5-g samples) were vacuum-packaged and irradiated as described previously and stored at -70°C for 2 to 3 days. Lipid oxidation was then measured according to the method of Ahn *et al.* (1998) with some modification. A 5-g sample was placed into a 50-mL centrifuge tube and homogenized with 20-mL ice cold distilled water by using a homogenizer (Virtishear, Virtis, Gardiner, NY) at a speed setting of 7 for 1 min. During homogenization, the tube was placed on ice. The homogenate was then filtered through a filter paper (2V, Whatman, Maidstone, Kent, UK). One-mL filtrate was then transferred to a 10-mL centrifuge tube, and 5-mL 7.2% butylated hydroxyanisole and 2-mL 0.65% thiobarbituric acid (TBA) in 20% trichloroacetic acid was added. The mixture was capped and vortexed, and incubated in boiling water for 20 min. Samples were cooled in running cold tap water for 5 min and centrifuged for 10 min at $2,500 \times g$. Supernatant was transferred to a 10-mL test tube and the absorbance at 532 nm was measured using a spectrophotometer (Sargent-Welch 6-550 UV/VIS, Pye Unicam Ltd., Cambridge, UK). The TBA reactive substance (TBARS) numbers were expressed as microgram malondialdehyde (MDA) per gram of meat.

Color Analysis

Bologna slices (4 mm) were placed in No. 400 Stomacher bags, overpacked in Mil-B foil bags, vacuum-packed to 0.26 mm Hg, and irradiated at 4°C as described above. Color analysis was then performed using a Hunter Lab Miniscan XE meter (Hunter Laboratory, Inc., Reston, VA) as outlined by Nanke *et al.* (1999). The meter was calibrated using white and black standard tiles. Illuminant A, 10° standard observer, and a 2.5-cm port/viewing area were used. Twenty readings were taken per parameter.

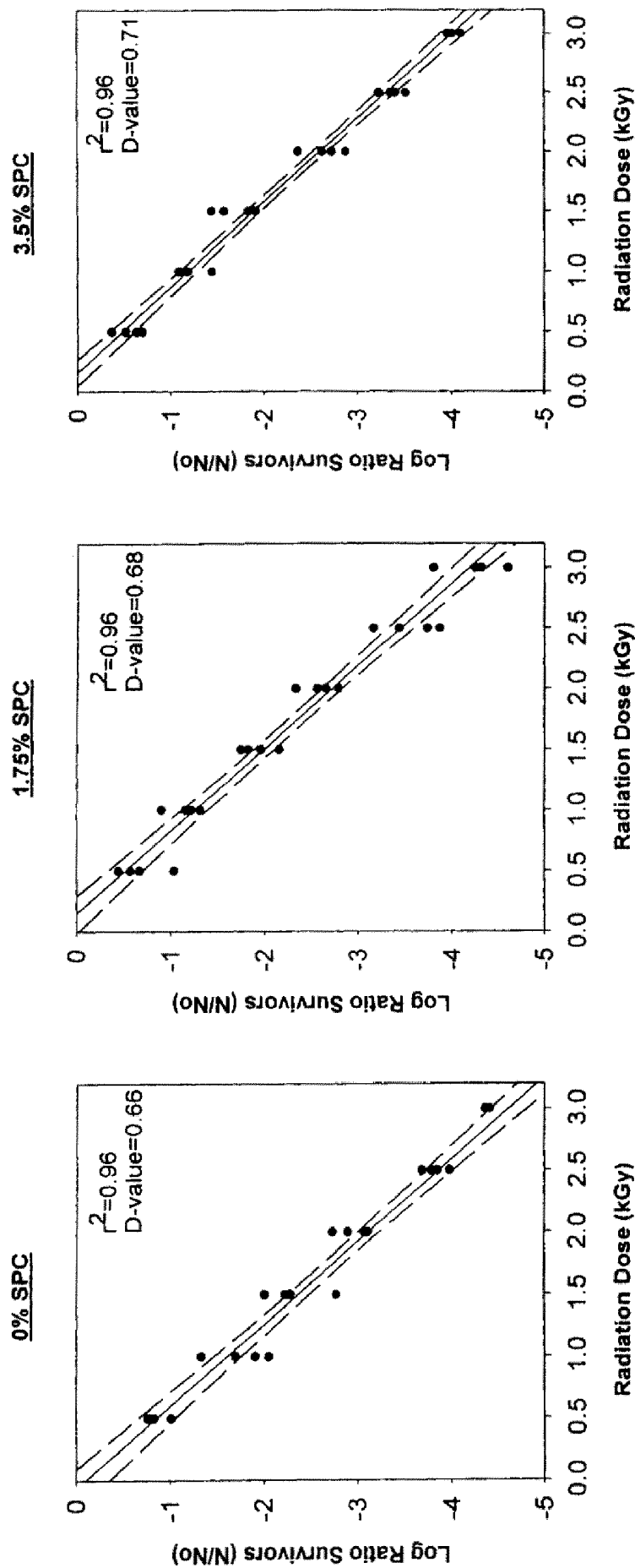


FIG. 1. RADIATION RESISTANCE OF *L. MONOCYTOGENES* COCKTAIL INOCULATED INTO BOLOGNA EMULSION CONTAINING 0, 1.75, OR 3.5% SPC

Log₁₀ reduction values are closed circles. Linear regressions are shown as solid lines with 95% confidence intervals shown as dashed lines. Each experiment was conducted independently four times.

TABLE 1.
LOG RATIO (N/N₀) REDUCTION DATA FOR *L. MONOCYTOGENES** IN BOLOGNA EMULSION CONTAINING SOY
PROTEIN CONCENTRATE (SPC)

	0.5 kGy	1.0 kGy	1.5 kGy	2.0 kGy	2.5 kGy	3.0 kGy	X-Intercept ^a
0% SPC	-0.85 (±0.06)	-1.75 (±0.16)	-2.32 (±0.016)	-2.93 (±0.08)	-3.83 (±0.07)	-4.74 (±0.21)	3.25
1.75% SPC	-0.68 (±0.13)	-1.15 (±0.09)	-1.93 (±0.09)	-2.59 (±0.10)	-3.72 (±0.10)	-4.25 (±0.17)	3.52
3.50% SPC	-0.55 (±0.07)*	-1.20 (±0.08)*	-1.69 (±0.11)*	-2.65 (±0.11)*	-3.37 (±0.06)*	-4.02 (±0.03)*	3.70

Log ratio survivors (N/N₀) with standard error in parenthesis.

*Statistically significant from 0% SPC control as determined by ANOVA (n=4, α=0.05).

^aX-Intercept at five log₁₀ reduction.

* Four strain *L. monocytogenes* cocktail (ATCC 7644, 15313, 43256 and 49594)

developed by Benzie and Strain (1998). Soluble antioxidant power of unirradiated emulsion increased significantly with increasing SPC concentration (Fig. 2). The antioxidant power was unaffected by irradiation as determined by ANOVA ($n=15$, $=0.05$). The lack of decrease in FRAP value may following irradiation may be due to lack of sensitivity of the assay. The increased antioxidant power and free radical scavenging ability of SPC at the 3.5% concentration may be responsible for the limited protection provided against the lethal effects of ionizing radiation observed for *L. monocytogenes* when suspended in cooked emulsion.

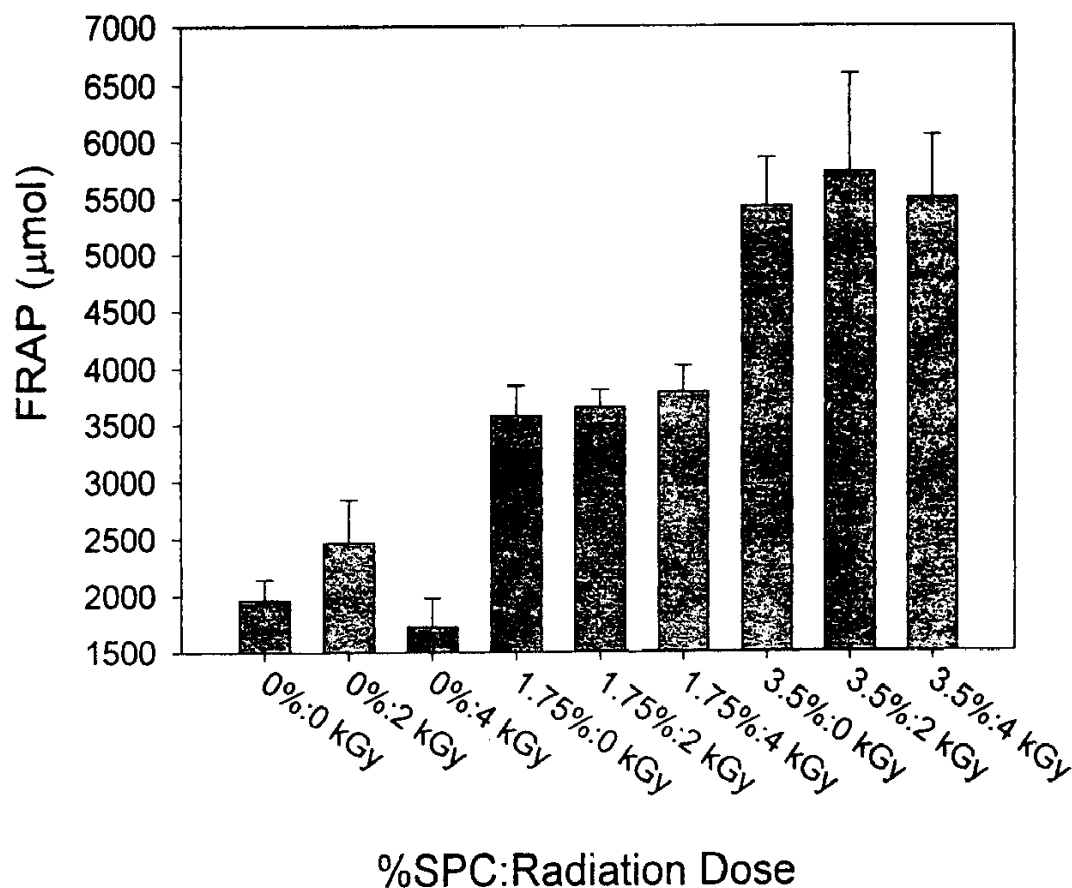


FIG. 2. ANTIOXIDANT POWER OF UNIRRADIATED AND IRRADIATED BOLOGNA EMULSION CONTAINING 0, 1.75, OF 3.5% SPC AS DETERMINED BY FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ASSAY

FRAP values are represented as μmol per gram emulsion antioxidant. The standard error bars ($n=15$) are included for each parameter.

Levels of lipid oxidation, as determined by TBARS assay and expressed in g/g malondialdehyde, were 1.20, 1.17 and 1.35 g/g in 0, 1.75, and 3.5% SPC emulsions, respectively (Fig. 3). The values were equivalent as determined by ANOVA ($n=5$, $=0.05$). Malondialdehyde concentrations increased, in a dose dependent manner, in the three product types as a result of irradiation (Fig. 3). SPC concentration, as a variable, did not protect the product against ionizing radiation-induced lipid oxidation.

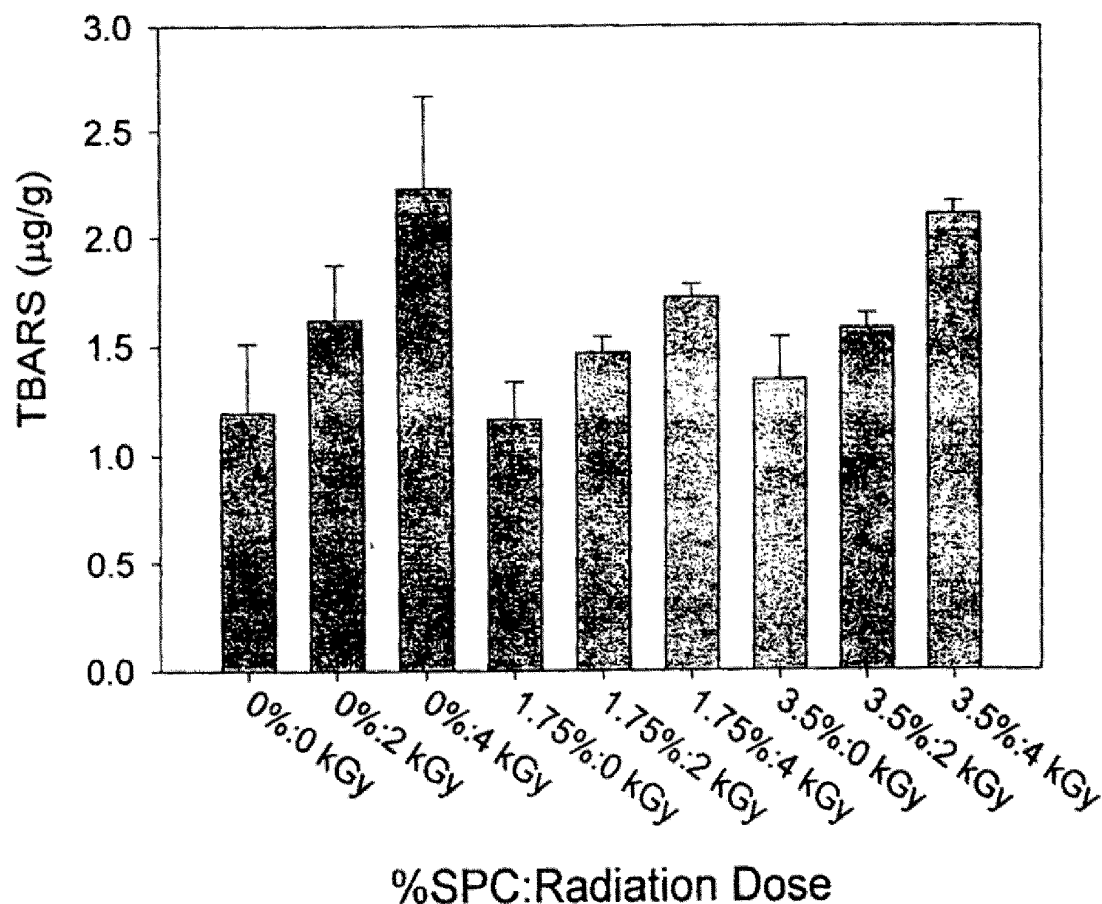


FIG. 3. LIPID OXIDATION IN UNIRRADIATED AND IRRADIATED BOLOGNA EMULSION CONTAINING 0, 1.75, OR 3.5% SPC AS DETERMINED BY THIOBARBITURIC ACID REACTIVE SUBSTANCE (TBARS) ASSAY

Values are represented in µg malondialdehyde per gram emulsion. The standard error bars (n=5) are included for each parameter.

Hunter-Color analysis was used to quantify color changes in 4-mm thick bologna slices containing 0, 1.75, and 3.5% SPC following irradiation to 0, 2.0, and 4.0 kGy (Fig. 4). ANOVA ($n=20$, $\alpha=0.01$) was used for statistical comparisons. SPC had no effect on L-value (brightness) of unirradiated bologna slices. A ionizing radiation dose-dependent decrease in L-value was observed as a result of irradiation in 0% SPC bologna ($p<0.01$) but not in 1.75 or 3.5% SPC bologna. Redness (a-value) was not effected by SPC in unirradiated bologna slices, but decreased in a dose-dependent manner as a result of irradiation regardless of SPC concentration ($p<0.01$). b-Value (yellowness) was greater in bologna containing SPC ($p<0.01$), probably due to the yellowish tint of the added SPC itself.

Yellowness decreased in 0 and 1.75% SPC bologna as a result of irradiation ($p<0.01$), but not in bologna containing 3.5% SPC. It should be noted that the b-value was greater at the 4-kGy doses for 1.75 and 3.5% SPC bologna than the unirradiated bologna with the same SPC concentrations. SPC reduced loss of brightness and yellowness, but not redness, in irradiated bologna containing SPC.

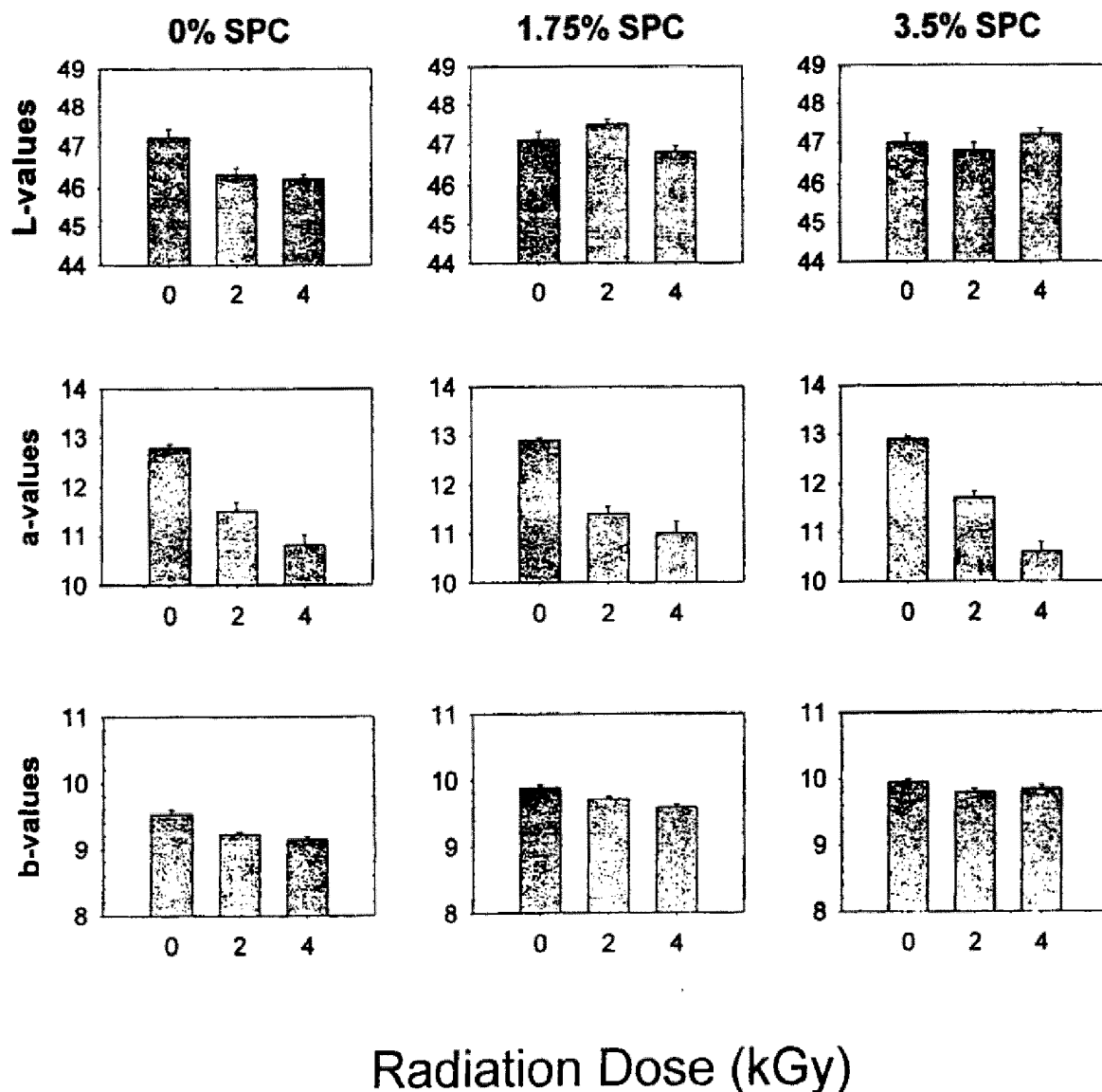


FIG. 4. COLOR ANALYSIS OF IRRADIATED AND UNIRRADIATED BOLOGNA SLICES CONTAINING 0%, 1.75% AND 3.5%

Standard error bars (n=20) are included for each parameter. a-Values represent product redness, b-values product yellowness, and L-values product brightness.

DISCUSSION

Water-soluble compounds including phenolics, flavonoids, and phytates contribute to soy protein concentrate associated antioxidant activity. SPC is allowed in cured fine emulsion sausages to a maximum concentration of 3.5% under current manufacturing guidelines (9CFR318.7). Inclusion of SPC in meats can delay lipid oxidation, preserve flavor, and extend product shelf-life (Ho *et al.* 1995; Wu and Brewer 1994; Romjin *et al.* 1991). We assessed the impact of SPC on cooked beef bologna emulsion containing 0, 1.75 and 3.5% SPC.

Ionizing radiation kills microorganisms by direct damage to genetic material or by the indirect effects of reactive oxygen species produced by the radiolysis of water on cell membranes and chromosomes (Ward 1991). The ability of ionizing radiation to eliminate foodborne pathogens can be attenuated by the presence of antioxidants. The antioxidants formate and polyethylene glycol protected *Salmonella typhimurium* against the effects of ionizing radiation when suspended in Butterfield's phosphate buffer (Kim and Thayer 1995). *L. monocytogenes*, when suspended in sodium erythorbate solution, is more resistant to ionizing radiation (Sommers *et al.* In Press). Spice extract solutions increased the radiation resistance of *Escherichia coli*, *Bacillus megaterium*, and *Bacillus pumilus* (Sharma *et al.* 2000).

Engeljohn *et al.* (1999) found that *Staphylococcus aureus* could be recovered from irradiated soy-fortified ground beef, but not nonfortified ground beef. El Wakeil *et al.* (1983a) found that high fat soy buffalo patties required a higher radiation dose to decrease microbial load as opposed to soy-less patties. Sommers and Thayer (2000) found that the D_{10} values for *L. monocytogenes* inoculated onto commercially available frankfurters ranged from 0.49 to 0.71 kGy, and speculated that the addition of binders and extenders with antioxidant activity could be partially responsible for that variation. At the 3.5% concentration (5494 mol antioxidant), SPC did provide limited protection against the lethal effects of ionizing radiation; however, the D_{10} values obtained and the radiation dose required to produce a five log₁₀ reduction in viable *L. monocytogenes* were similar to the range reported by Sommers and Thayer (2000).

Inclusion of soy protein concentrate, in concentrations commonly used in cooked and cured ready-to-eat meat emulsions, did not prevent ionizing radiation-induced lipid oxidation. Lipid oxidation, as determined by TBARS assay, increased in 0, 1.75 and 3.5% SPC containing bologna emulsion in a radiation dose-dependent manner. Lipid oxidation, in both full fat and defatted soy meal, has been shown to increase in a radiation dose-dependent manner (Horvatic and Gruener 1993; Gruener *et al.* 1992). El Wakeil *et al.* (1983b) found that lipid oxidation increased in a dose-dependent manner in buffalo meat/soy patties. SPC, while increasing soluble antioxidant power, does not prevent ionizing radiation-induced lipid oxidation.

Ionizing radiation can induce loss of color in meat products (Ahn *et al.* 1999; Chen *et al.* 1999; Nanke *et al.* 1998, 1999). In this study SPC did not protect beef bologna against ionizing radiation-induced loss of redness (a-value), but did prevent ionizing radiation-induced loss of yellowness (b-value) and brightness (L-value). Commercially available fine emulsion sausages vary considerably in formulation and appearance of the final product. While side-by-side comparison revealed the loss of redness in the bologna slices, these authors would consider those changes acceptable based on the appearance (color) of other commercially available cured meat products.

SPC is commonly used in the manufacture of ready-to-eat meat products including bologna and frankfurters. While minor changes were observed in the radiation resistance of *L. monocytogenes*, lipid oxidation, and product color the results indicate that SPC would not represent a barrier to ionizing radiation pasteurization of ready-to-eat meat products for the parameters examined in this study.

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